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EVALUATION OF ANALGESIC PROPERTY OF *DESMODIUM VELUTINUM* (P.BEAUV.) DC (PAPILIONACEAE)

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ABSTRACT: *Desmodium velutinum (family Papilionaceae) is used in Nigeria and Lokoja in particular for the treatment of abdominal pain among others. The LD_{50} was evaluated along with analgesic properties. The analgesic effects of the methanol extract of D. velutinum were investigated at three dose levels (100, 200, 300) mg/kg on the experimental models of pain in mice. The result of LD_{50} was greater than 3000mg/kg showing D. velutinum extract is relatively safe for human consumption. The anti-nociceptive activity was evaluated using the hot-plate and abdominal constriction tests. The extracts produced significant ($P<0.05$) inhibition of thermal nociception induced by hot plate. On chemical nociception induced by intra-peritoneal acetic acid, the extracts significantly ($P<0.05$) decreased the number of writhing episodes and the time spent before jumping off the hot-plate in a dose independent manner. These results suggest that the extract of D.velutinum may act by inhibiting the mediators of pain. These findings may justify the use of the plant in traditional medicine in the management of pain and related diseases in Nigeria.*

KEYWORDS: Evaluation, Anlgesic, Activity, Desmodium Velutinum, Papilionaceae

INTRODUCTION

Pain is an unpleasant sensation that is often associated with actual or potential tissue damage that is caused by disease and/ or injury. The word "pain" comes from the latin word "peona" meaning a fine or penalty (Coda and Bonica, 2001). The management of pain and related problems is a real challenge that people face daily. Although several drugs are available for this condition, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic agent (Sayyal, *et. al*, 2004). Throughout history, man has used many different forms of therapy for the relief of pain. Among them, medicinal herbs are highlighted due to their wide popular use. Examples include *Papaver somniferum* from which morphine and codeine were isolated and *Cannabis sativa* from which cannabinoid was isolated. Morphine is regarded as the prototype of opiate analgesic drugs. In the relief of pain, opiates are generally considered to act on the central nervous system (CNS) exercising their effects through opioid receptors (Klawe and Maschke, 2009). Although morphine has reigned for centuries as the king of painkillers, its rule has not been totally benign (Anonymous, 2009).

D. velutinum is a medicinal plant used for treating/managing abdominal pain, general body pain and fever. This plant is found in wooded grassland in Lokoja, Nigeria. However, up till date, there is no scientific report or verification of the use of this plant in pain management. This study investigated the analgesic activity of the methanol extract of *D. velutinum* in mice.

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MATERIALS AND METHOD

Collection and Identification of the Plant Sample

The plant sample was collected and identified at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria - Nigeria.

Extraction for Analgesic Activity

The plant was air-dried and powdered using pestle and mortar. About 250g of the powdered material were macerated in 500ml of 70% methanol for 24 hours. The extract was concentrated on a water-bath.

Experimental Animals

Swiss albino mice (18-20g) of both sexes were used and maintained on standard animal feed (Excel Feed$^{(R)}$, Ilorin-Nigeria) and water *ad libitum* in the Animal House, Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria.

Acute Toxicity Studies

The method described by (Lorke, 1983) was adopted. Thirteen mice were used. In the first phase, 3 different doses (10, 100, 1000 mg/kg) were administered to 3 groups each containing 3 mice. In the second phase, more specific doses of 1600, 2900 and 5000 mg/kg were administered to 3 groups, each containing one mouse. The median lethal dose (LD_{50}) value was obtained as a geometric mean of the highest non-lethal dose and the lowest lethal dose.

Hot Plate Method

The method as described by Raquibul *et al.*, (2010) was adopted. Five groups each containing 5 mice were used. The three different doses used in this procedure were determined from the acute toxicity studies. The first group served as **negative** control and received normal saline of 10mg/kg. The second group was administered with morphine as the standard drug (20mg/kg) which served as **positive** control while the other 3 groups received extract at 3 different doses of 100, 200, 300 mg/kg respectively as **test** doses. All administrations were by intra-peritoneal route. The tests were carried out using Eddy's hot plate apparatus. The temperature was set at 55 ± 1°C. Each mouse was placed on the hot plate and the reaction time in seconds for flicking of hind paw or jumping at 0 second, 30 minutes, 60minutes, 90 minutes and 120 minutes with cut off time of 15seconds was recorded. The reaction time following the administration of the test extracts, reference standard drug, and control saline vehicle were recorded.

Acetic Acid-Induced Writhing Method

The method as described by Raquibul *et al*., (2010) was adopted. Five groups each containing 5 mice were used. One group, the negative control, received normal saline (10mg/kg). Another group, the positive control received pentazocine as the standard drug (20mg/kg). The remaining 3 groups, the test groups received 3 different doses (100, 200, 300mg/kg) of extract. Each group was treated 30 minutes before the administration of 0.6% acetic acid. After an interval of 5 minutes, the mice were observed for abdominal contraction referred to as writhing for the next 10 minutes.

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Statistical Analysis

The results obtained from the above were statistically analyzed using analysis of variance (ANOVA).

RESULTS

Acute Toxicity Studies

Mice treated with doses of 10, 100 and 1000 mg/kg for the phase 1 of the experiment did not show any sign of toxicity or death after 24 hours. From the second phrase, the mouse which was administered with a dose of 5000mg/kg died after 24 hours. This showed that the crude drug is to some extent toxic. The LD_{50} was the taken as the geometric mean of the lowest lethal dose and the highest non-lethal dose giving 3807.

Acetic Acid-Induced Writhing Test

Results were expressed as mean ± standard error of mean (SEM).

Table 1: Effects of Methanol Extract (ME) of the Leaf of *D. velutinum* on Acetic Acid Induced Writhing in Mice

S/N	**Treatment (mg/kg)**	**Mean number of Writhings**	**% Inhibition**
1	Normal saline (10)	42.80 ± 3.96	0.00
2	Pentazocine (20)	21.80 ± 1.27	49.07
3	ME (100)	23.40 ± 2.15	45.32
4	ME (200)	24.60 ± 2.71	42.52
5	ME (300)	27.20 ± 3.34	36.62

n = 5

Hot Plate Method

Table 2: Effect of Methanol Extract (ME) of the Leaf of *D. velutinum* on Hot Plate Induced Pain in Mice

S/N	**Treatment in mg/kg**	**0 min**	**30min**	**60min**	**90min**	**120min**
1	Normal saline (10)	1.4	1.4	1.6	1.2	1.4
2	Morphine (20)	2.2	4.6	3.6	3.4	3.0
3	ME (100)	2.0	3.4	3.2	2.4	1.4
4	ME (200)	1.4	2.8	2.2	1.6	1.4
5	ME (300)	1.4	3.0	2.2	1.4	1.2

n = 5

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DISCUSSION

The LD_{50} (>3000mg/kg) obtained from the leaf methanol extract of *D. velutinum* showed no observed side effect which probably suggests that the plant extract is relatively safe for mice. It is a great advantage for any plant extract used for herbal treatment to have a very low toxicity, i.e. high LD_{50}. According to Clarke and Clarke, (1979), any substance whose LD_{50} is higher than 1000mg/kg is considered relatively safe.

The number of writhing in mice at 300 mg/kg of the extract (27.20±3.34) was significantly lower ($P \leq 0.05$) when compared with that obtained with the normal saline (42.80±3.96) while at 100mg/kg of the extract (23.40 ± 2.15) was even significantly much more lower when compared to the normal saline showing a dose response relationship. However, there is statistically significant difference ($P \leq 0.05$) in the number of writhing in the mice administered with standard drug (21.80±1.27) and normal saline (table 1). Similarly, the percentage inhibition of pain in the mice treated with the extract is higher than that treated with normal saline. The standard drug gave the highest inhibition among the experimental groups. The acetic acid-induced writhing method showed that at lowest dose, it gave the highest analgesic properties which were comparable with those of pentazocine, a peripherally acting analgesic.

The acetic acid-induced writhing is a visceral pain model that has been associated with release of arachidionic acid, cyclooxygenase (COX), bradykinins and substance P which induce pain. Prostaglandin biosynthesis plays a role in nociceptive mechanisms (Satyanarayana *et al.*, 2004). Therefore this model of pain might have been inhibited by peripheral analgesics through the inhibition of COX activity. The results show that lowest dose of 100mg/kg has peripheral analgesic properties similar to pentazocine by inhibiting the release of endogenous pain mediators.

The number of seconds expended by mice before jumping off the plate at a dose of 300mg/kg was significantly higher ($P \leq 0.05$) when compared with that obtained in the normal saline. So also, the number of seconds expended by mice before jumping off the hot plate at a dose of 100mg/kg was significantly higher than normal saline. However, there is statistically significant difference at ($P \leq 0.05$) in the number of seconds expended by mice administered with standard drug (morphine) when compared with normal saline (table 2). The hot-plate test was utilized to assess the central anti-nociceptive properties of *D. velutinum* leaf extract. This test is sensitive to drugs which exert their analgesic effects through the central nervous system (CNS). At doses of (100, 200 and 300mg/kg), the extract significantly ($P<0.05$) increased reaction time to thermal stimulation from 30 to 120 minutes post treatment. The extract and morphine exhibited similar increase in activity at 30 and 60 minutes of administration and later there were decrease in activity at 90 and 120 minutes. It was also observed that at lower dose, higher activity was observed. This is in line with what is called "carrier mechanism" a phenomenon that explains the zero increase in the activity of some drugs as dose is increased (Sayyah *et al*., 2004). Morphine, a central acting analgesic had increased reaction time from 30 minutes post treatment. Since the extract was able to increase the latency to thermal stimulation, it could also suggest that the extract possesses some central analgesic properties. Although the exact chemical compound or compounds responsible for the analgesic effect of the leaf extract of *D. velutinum* are not known, the observed pharmacological effects may have been due to a single constituent or by the complex interaction among the various chemical constituents.

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The leaf extract showed anti-nociceptive and analgesic properties. The results of the analgesic activity tests were expressed as mean ± standard error of mean (SEM). Analysis of Variance (ANOVA) test was used to analyze the data. Comparison between control and drug treated groups were considered to be significant ($P<0.05$). The results of the extract on pain (tables 1 and 2) were compared based on the effect of the number of writhing produced by acetic acid induced writhing method and the hot plate in the hot plate induced pain method along with negative control (normal saline) and positive control (standard drug) in mice.

CONCLUSION

This research is the first of its kind on *D. velutinum* used for pain management/treatment. *D. velutinum* has been evaluated for its macroscopy, microscopy, physical constant, chromatography and biological activities. The findings in this research have provided useful information for a monograph of the plant and have validated the traditional use of the plant for pain management/treatment.

REFERENCES

Anonymous (2009). Cancer Pain Relief and Palliative Care; Report of WHO Expert Committee. *World Health Organization Technical Report Series*, 804. Geneva, Switzerland: World Health Organization. pp. 1–75.

Clarke, E. G. C. and Clarke, M. L. (1979) *Veterinary Toxicology*, Bailiere Tindall Ltd, London, 1st Edition, p10.

Coda, B.A. and Bonica, J.J (2001). *General Consideration of Acute Pain*. Bonica J..J Bonica's Management of Pain (3rd ed.). pp 207-216.

Lorke, D. (1983). *A New Approach to Practical Acute Toxicity Testing*. Archieves of Toxicology, Germany, 54: 275 - 287.

Klawe, C. and Maschke, M. (2009). "Flupirtine: Pharmacology and Clinical Applications of a Nonopioid Analgesic and Potentially Neuroprotective Compound". *Expert Opinion on Pharmacotherapy* 10 (9): 495–500.

Raquibul, S. M., Husain M.M., Akter, M.J., Mazunder, M.E.H., Alam, M.A., Faruque, A., Rana, S. and Rahma, S. (2010). Analgesic Activities of Different Fractions of The Aerial Parts Of *Commelina benghalensis* Linn, *International Journal of Pharmacology*. 6(1): 63-67.

Satyanarayana, P.S.V., Jain N.K., Singh, S. and Kulkarni, S.K. (2004). Effect of Selective Inhibition of Cyclooxygenase-2 on Lipopolysaccharide induced Hyperalgesia. *Inflammopharmacology*. 12: 57–68.

Sayyah M, Hadidi N. and Kamalinejad M. (2004) Analgesic and Anti-inflammatory Activity of *Lactuca Sativa* Seed Extract in Rats. *Journal of Ethnopharmacology*. 92: 325–329.

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